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Light-Sensitive Voltage Responses in the Neurons of the Cerebral Ganglion of *Ciona savignyi* (Chordata: Ascidiacea)

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Light-responsive behaviors such as siphon contraction (1), phototropism (2), and gamete release (3, 4) have been described in several ascidian species. The pigmented spots around the siphon openers (5), the epithelial cells of the sperm duct (6, 7), and the cerebral ganglion (8) have been suggested to be the photoreceptor candidates underlying these behaviors. However, these arguments have not yet been settled because no direct electrophysiological recordings of light-induced receptor potentials have been reported. In this study, we focused on the cerebral ganglion and performed intracellular recordings from the neurons in the ventral side of the cerebral ganglion in an isolated in vitro *preparation of the neural complex in* Ciona savignyi. *We found that 24% (n = 115) of the recorded neurons showed various types of voltage responses to light stimuli. Almost all (27/28) of the recorded voltage responses were "on" responses that included hyperpolarizing and depolarizing responses and could be categorized into five types, except for a complex response recorded in one cell; the remaining one (1/28) was a depolarizing "off" response. This is the first report of electrophysiological recordings of light-sensitive voltage responses from ascidian cerebral ganglion neurons.*

Membrane potentials were recorded with intracellular microelectrodes from 115 neurons in the ventral side of the isolated cerebral ganglion of 47 ascidians (see Fig. 1 legend for detail). The resting membrane potential was − 48 ± 12 mV (mean ± SD, $n = 52$), and 52% ($n = 115$) of the recorded neurons showed spontaneous activity consisting of low-frequency (0.1–0.3 Hz) regular discharges of action potentials (Fig. 1B, D). Seventeen percent of the neurons showed spontaneous irregular or bursting discharges of action potentials. The remaining 30% of the neurons were silent (Fig. 1A, C, E). In addition, 24% (28 cells) of the total 115 recorded neurons, including spontaneously active and silent ones, showed voltage responses of various types to light stimuli (Fig. 1). Most of these light responses (27/28 cells) were "on" responses (Fig. 1A–E), and we observed a transient "off" depolarization with a few spikes in only one neuron (not shown). Twenty-six out of the 27 "on" responses could be categorized into five types as follows (Fig. 1): (A) transient hyperpolarization (13 cells); (B) suppression of spontaneous discharge of action potentials (7 cells); (C) transient depolarization (2 cells); (D) transient high-frequency excitatory synaptic inputs (2 cells); (E) sustained depolarization (2 cells). The remaining "on" response consisted of a complex pattern: transient hyperpolarization followed by sustained depolarization accompanying a number of spikes during the light stimulus. This was similar to a mixture of response types A and E. There seemed to be no significant differences in the resting potentials for the five response types. Even the quickest of the five types of responses, type A, showed a latency longer than 500 ms, and the latency of the other types was 3–5 s. There seemed to be no specific distribution of these types of neurons on the ventral surface of the cerebral ganglion. The ability of a cell to respond with more than one response type when presented different light intensities or wavelengths was not investigated.

Hyperpolarizing receptor potentials of the visual cells in the ocellus of ascidian larvae has been described previously (9). Because our type A and B responses also are hyperpolarizing, it is tempting to interpret them as receptor potentials. However, there is also a significant difference between

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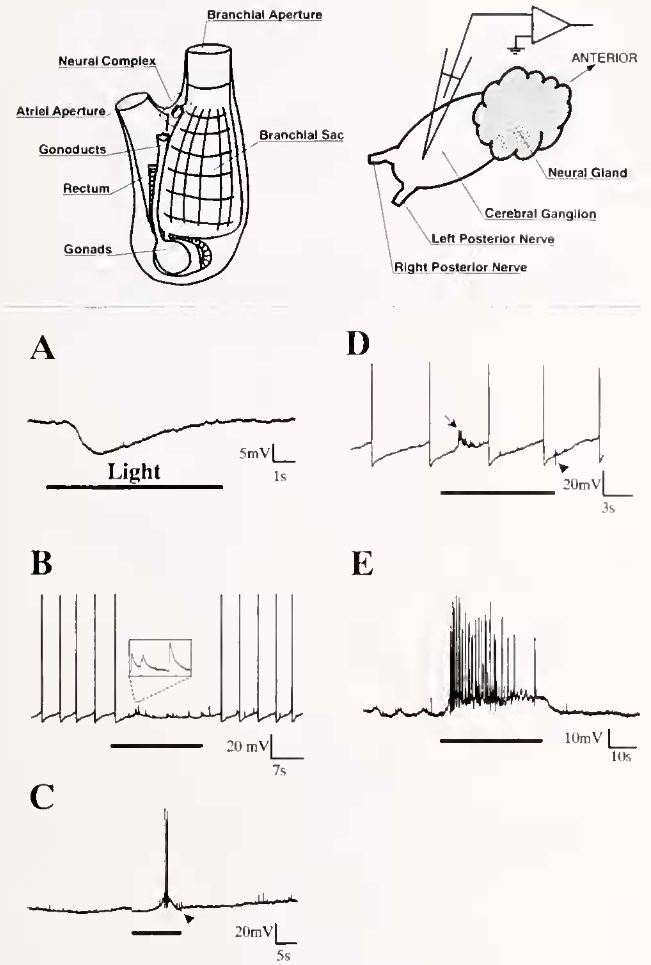

Figure 1. (Top left) Schematic illustration of adult *Ciona savignyi* showing location of major organs. The illustration was modified from Mackie (13). Neural complex is located in the body wall between the two siphons (boxed). (Top right) Ventral view of the isolated neural complex preparation. The neural complex consists of the cerebral ganglion and the neural gland. Recordings were made from neurons of the ventral side of the cerebral ganglion. (Bottom) The 27 "on" voltage responses to light stimuli could be categorized into five types, except for one complex response (see text). The typical response for each type is shown. (A) Transient (<5 s) hyperpolarization (13/27). Longer or continuous light stimulation did not evoke any additional voltage changes after the transient hyperpolarization. (B) Suppression of spontaneous regular discharges (7/27). Small irregular fluctuations of membrane potential indicative of synaptic inputs seemed to increase in frequency during light stimuli. Some of them are enlarged in the inset corresponding to 1 s × 10 mV. (C) Transient depolarization accompanied by a few spikes (2/27). Longer or continuous light stimulation did not evoke any voltage changes after the transient depolarization. (D) Transient high-frequency excitatory synaptic inputs (arrow), which sometimes build up to give rise to an action potential in spontaneously beating neurons. Unlike the type B response, the spontaneous regular discharges were not affected in these cells (2/27). (E) Sustained depolarization during light stimuli, accompanied by a number of spikes with frequency accommodation (2/27). Voltage responses of each neuron against repeated light stimuli were reproducible during the recording period. Arrowheads in C and D indicate stimulus artifacts.

Methods: *Ciona savignyi* was used for the present electrophysiological study because of the advantageous morphology of its neural complex (composed of closely apposed cerebral ganglion and neural gland). The neural gland of this species is located in the anterior ventral part of the cerebral ganglion (Tsutsui and Oka, unpubl. obs.), and a large ventral surface area of the cerebral ganglion is easily accessible *in vitro* with microelectrodes. The neural complex was dissected out, and muscular tissue and the thin wall of the blood sinus that cover the ventral side of the cerebral ganglion were gently removed. Then, the isolated neural complex preparation was pinned ventral side up in a recording chamber (volume = 400 μl) lined with Sylgard and was perfused with filtered seawater (1 ml/min). The experiments were carried out at room temperature (18–22° C), and the preparation was viable at least for 6 h under these conditions. The neurons on the ventral side of the posterior part of the cerebral ganglion were recorded intracellularly by sharp microelectrodes (pulled from borosilicate glass of o.d. 1.5 mm with inner filaments and filled with 2 *M* KCl; resistance = 40–70 MΩ) under a dissecting microscope. Signals were amplified (by MEZ-8300, Nihon Kohden) and digitized at 3–5 kHz and stored digitally (using Axotape software, Axon Instruments). Light stimuli (3.0×10^4 lux) were delivered to the preparations through an optic fiber from a conventional light source of a 150 W halogen bulb (type 6423, Philips) equipped with a heat-absorbing filter. The light stimulus was controlled manually by a solenoid relay switch. The preparation was kept in the dark (<0.1 lux) for at least 1 min between the light stimuli.

the two; the receptor potential of the larva is fast and sustained, while the type A and type B responses recorded in the present study are slow and transient. We cannot conclude at present which type of response represents the photoreceptor potential, because chemical and electrical synaptic transmissions were not blocked in the present recordings. Actually, the increase in frequency of synaptic inputs in the type D response is a second-order or higher order response that arises from synaptic pathways. However, because all of these responses were recorded from neurons within 'isolated' cerebral ganglion preparations, it is evident that there should be a certain population of photoreceptor cells within the cerebral ganglion of *Ciona savignyi*.

Light-induced gamete release is a well-known behavior of ascidians (3, 4). However, removal of the whole neural complex *in vivo* does not affect this behavior in *Ciona intestinalis* (Tsutsui and Oka, unpubl. data). Similar behavior was reported previously in *Chelyosoma productum* (10). These results may support the existence of an ascidian photoreceptive system or systems other than the cerebral ganglion. It may be an interesting future study to compare the action spectra of the voltage responses of the light-sensitive neurons in the present cerebral ganglion preparation and those of light-induced spawning behavior, such as that measured by Lambert *et al.* (3). Such a comparison would test whether similar photoreceptor processes underlie the control of spawning as well as the voltage responses reported here and would further our understanding of the properties of ascidian photoreceptive systems.

The cerebral ganglion of ascidians has been shown to have many neurons immunoreactive for vertebrate neuropeptides such as GnRH, bombesin, and neurotensin (11, 12). Especially, tunicate GnRHs have been biochemically characterized, and the distributions of GnRH neurons have been studied recently (11, 13, 14). The recordings of light-sensitive voltage responses in the present study were made mainly from the posterior ventral part of the cerebral ganglion, the region where substantial numbers of immunoreactive GnRH neurons are distributed in *Ciona intestinalis* (11) and *C. savignyi* (Tsutsui and Oka, unpubl. data). Therefore, the present results show that light-sensitive neurons and GnRH-secreting neurons are located in an overlapping region, and it may be suggested that GnRH release is controlled by light. This possibility is interesting from the viewpoint of the general neuromodulatory functions of GnRH neurons (15, 16). The ascidian cerebral ganglion preparation may, therefore, provide a chance to test this hypothesis.

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